IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	YAMAOKA ET AL.	Examiner:	M. MEAH
Serial No.:	10/526026	Group Art Unit:	1652
Filed:	February 28, 2005	Docket No.:	10921.0286USWO
Title:	METHOD FOR PURIFYING PROTEIN AND GLUCOSE DEHYDROGENASE		

CERTIFICATE OF TRANSMISSION.
I hereby certify that this paper is being transmitted by EFS Web to the United States Patent & Trademark Office on March 31, 2010.
By: /Melissa Zarns/
Name: Melissa Zarns

APPELLANTS' BRIEF ON APPEAL UNDER 37 C.F.R. §41.37

Mail Stop Appeal Brief-Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

This Brief is presented in support of the Notice of Appeal filed February 1, 2010, from the final rejection of Claims 1, 6-9, 11-14, and 24-26 in the above-identified application, as set forth in the final Office Action mailed September 1, 2009.

Please deduct the amount of $540.00 from Deposit Account No. 50-3478 for the required fee for filing this Brief under 37 C.F.R. §41.20(b)(2).

I. REAL PARTY OF INTEREST

The real party of interest is ARKRAY, Inc. of Kyoto, Japan who is the assignee of the above-identified application. An assignment was recorded on August 11, 2005 under reel/frame 016633/0980.

II. RELATED APPEALS AND INTERFERENCES

The Appellants, Assignee, and the Assignee's legal representatives are unaware of any other appeals, interferences, or judicial proceedings that will affect, be directly affected by or have a bearing on the Board's decision in this Appeal.

III. STATUS OF CLAIMS

Claims 1, 6-9, 11-14, and 24-26 are pending and are the subject of this Appeal. Claims 1, 6-9, 11-14, and 24-26 were present in the Listing of Claims of the Amendment filed on June 9, 2009. The pending claims were entered and considered in the final Office Action dated September 1, 2009. Claims 2-5, 10, and 15-23 have been canceled. A copy of the pending claims 1, 6-9, 11-14, and 24-26 is provided in the appendix at section VIII of this Appeal. For purposes of this appeal only, claims 1, 6-9, 11-14, and 24-26 stand or fall together.

IV. STATUS OF AMENDMENTS

An Amendment was filed on June 9, 2009. The Amendment was entered and considered in the final Office Action mailed September 1, 2009, where the claims and remarks were not considered to place the application in condition for allowance.

V. SUMMARY OF CLAIMED SUBJECT MATTER

Appellants' invention resides in features of claims 1 and 7, which are summarized below.

Claim 1

Claim 1 is directed to a method of purifying a target protein from a protein solution containing the target protein by using liquid chromatography. (See e.g. page 3, lines 4-6.) The target protein is glucose dehydrogenase (GDH) derived from a microorganism belonging to the genus *Burkholderia* and has α, β, γ subunits. (See e.g. page 3, lines 24-25 and page 5, line 11 to page 6, line 9). Claim 1 comprises two steps. The first step is introducing the protein solution into a column filled with a packing agent, the packing agent holding the target protein, and being an ion-exchange resin containing a quaternary ammonium group as an ion-exchange group. (See e.g. page 3, lines 7-9 and page 4, lines 1-4.) The second step is eluting the target protein by using an eluent containing a cholate. (See e.g. page 3, line 10 and page 4, line 15.)

Appellants have discovered that, by using an ion-exchange resin that has a quaternary ammonium group as the particular packing agent in combination with a cholate as the particular eluent, the specific target protein GDH derived from *Burkholderia* can be purified more efficiently, resulting in better collections and significantly higher specific activity of the protein. (See e.g. Discussion of Results on page 27, line 12 to page 28, line 8.)

Claim 7

When the eluent is a cholate that specifically comprises sodium cholate as recited in claim 7 (see e.g. page 4, line), such purification benefits as noted above have been realized. (See e.g. Examples 1-4 compared to Comparative Examples 1 and 2, and results reported in Tables 2-7 and Figs. 1-3.)

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

Whether Shimomura et al., Analytical Biochemistry, vol. 153, 126-131(1986) (hereafter "Shimomura et al.") in view of Sode, WO 02/36779, English Translation in US 2004/0023330 (hereafter "Sode"), and further in view of Amersham catalog 1999, pages 520, 523, and 527 (hereafter "Amersham"), render claims 1, 6-9, 11-14, and 24-26 obvious under 35 U.S.C. §103(a), and more particularly:

A. Whether there is any suggestion or motivation to modify or combine the teachings of Shimomura et al. with the teachings of Sode and Amersham, as alleged in the rejection of claims 1, 6-9, 11-14, and 24-26.

B. Whether there is any reasonable expectation of success to lead one of skill in the art to claims 1, 6-9, 11-14, and 24-26, based on the teachings of Shimomura et al., Sode, and Amersham and not based on Appellants' disclosure.

VII. ARGUMENT

Claims 1, 6-9, 11-14, and 24-26 are not obvious under 35 U.S.C. §103(a) in view of Shimomura et al., in view of Sode, and in view of Amersham.

Claims 1, 6-9, 11-14, and 24-26 were rejected under 35 U.S.C. 103(a) as being unpatentable over Shimomura et al., in view of Sode, and in view of Amersham. Appellants respectfully request reversal of the rejection, because a prima facie case of obviousness has not been shown for the following reasons.

A. There is no suggestion or motivation to modify or combine the teachings of Shimomura et al. with the teachings of Sode and Amersham.

In order for references to be combined in an obviousness rejection, there must be a source of motivation to modify the teachings of one reference using the teachings of other references. In *KSR International Co. v. Teleflex Inc.*, the Supreme Court reconfirmed that obviousness is not proved merely by demonstrating that each of its elements was, independently, known in the prior art. 550 U.S. 398, 419 (2006). The Court recognized that when there is a design need or market pressure to solve a problem and there are a finite number of identified predictable solutions, a person of ordinary skill in the art has good reason to pursue the known options within his or her technical grasp; however, the Court also reconfirmed the importance of identifying a reason that would have prompted a person of ordinary skill in the art in the relevant field to combine the elements in the way the claimed invention does. Id. at 417, 418. Further, there may not be an inference of obviousness in technologies with unpredictable solutions and that do not have a small and finite number of alternatives. *See Ortho-McNeil Pharmaceutical, Inc. v. Mylan Laboratories, Inc.*, 520 F.3d 1358, 1366, 86 U.S.P.Q.2d 1196, 1202 (Fed. Cir. 2008). Rather, the Federal Circuit has concluded that it is not proper to simply retrace the path of the inventor, and to discount the number and complexity of alternatives to arrive at the invention. *Id.* at 1366, 1202.

Claim 1 is directed to a method for purifying a target protein from a protein solution containing the target protein by using liquid chromatography. The target protein is glucose dehydrogenase derived from a microorganism belonging to the genus Burkholderia and has α, β, γ subunits. The liquid chromatography comprises a first step of introducing the protein solution into a column filled with a packing agent. The

packing agent holds the target protein, and is an ion-exchange resin containing a quaternary ammonium group as an ion-exchange group. In a second step, the target protein is eluted using an eluent containing cholate.

There is no suggestion or motivation to combine Shimomura et al. with Sode and Amersham so as to render claim 1 obvious.

Shimomura et al. is directed to purification of Complex III (cytochrome bc_1 complex) under a rechromatography approach (same column) to serially elute iron-sulfur protein, cytochrome c_1, and ubiquinone-binding protein, by changing elution buffers (see Abstract on page 126). Shimomura et al. discusses use of an eluent containing cholate, but the reference fails to disclose or suggest the claimed specific glucose dehydrogenase (from *Burkholderia*). Shimomura et al. also fails to teach or suggest the specific ion-exchange resin, namely an ion-exchange resin containing a quaternary ammonium group as an ion-exchange group.

Sode discusses glucose dehydrogenase from the genus *Burkholderia* having α, β, and γ subunits (see paragraph [0165-0166]. However, Sode fails to disclose or suggest elution using a cholate. As with Shimomura et al., Sode does not teach or suggest chromatography using an ion-exchange resin containing a quaternary ammonium group as an ion-exchange group, which is also acknowledged in the final Office Action.

Appellants acknowledge that Amersham discloses the availability of Q sepharose fast flow columns (see page 527), having an ion exchange resin containing a quaternary ammonium group as an ion-exchange group (see page 523).

However, Appellants respectfully submit that claim 1 does not follow from the references cited, because there is no reasonable suggestion or motivation for one of skill

in the art to combine the references. While the references individually mention glucose dehydrogenase from *Burkholderia* (Sode), an eluent containing cholate (Shimomura et al.) and a packing agent (Amersham), the record provides no reasonable suggestion that one of skill in the art would select, or receive any direction to select, the particular eluent and packing agent for purifying the particular target protein required by claim 1.

There is no suggestion or motivation in the art to use an eluent containing cholate for purifying protein glucose dehydrogenase derived from *Burkholderia*, much less combine an eluent containing cholate with the specific packing agent (i.e. having an ion exchange resin containing a quaternary ammonium group). Shimomura et al., which is relied upon for teaching a cholate eluent, uses not only deoxycholate with sodium chloride, but uses other eluents containing guanidine hydrochloride and sodium dodecyl sulfate (see e.g. page 127). With further reference to Shimomura et al., the article does not identify anything special about selecting an eluent containing cholate, and in fact reports that further chromatography (e.g. using a cholate eluent) is needed to purify certain fractions after its rechromatography approach due to the presence of other protein contaminants (see page 128 left column). Regarding Sode, the reference only uses sodium chloride as an eluent, and nowhere in Sode is there any suggestion to use eluents other than different gradients of sodium chloride (see Purification of Enzyme, paragraphs [0172] to [0175]. There is nothing in Shimomura et al. to suggest to one of skill in the art to select such an eluent for combination with the packing agent and for purifying glucose dehydrogenase derived from *Burkholderia* as claimed.

Likewise, there is no suggestion or motivation in the art to use an ion exchange resin containing a quaternary ammonium group as the particular packing agent in the

purification of protein glucose dehydrogenase derived from *Burkholderia*. The mere fact that packing agents having an ion exchange resin containing a quaternary ammonium group were available in the catalog by Amersham, as alleged by the final Office Action, does not rise to an inference that such packing agents would be desirable for purifying protein glucose dehydrogenase derived from *Burkholderia*, or to an inference that such packing agents would be desirable for use with an eluent containing cholate. Further, the fact that these elements were independently present in the art does not alone provide a suggestion or motivation to combine them, especially when there is not necessarily a finite number of combinations or any predictability as to how such combinations would respond. Nothing in the record provides a reasonable justification for making such a combination as alleged, or for modifying the references to lead to claim 1. Consequently, claim 1 does not follow from the references.

For the foregoing reasons, Appellants respectfully contend that a prima facie obviousness has not been shown, as a suggestion or motivation to combine or modify the references has not been shown. Claim 1 and its dependents are patentable over the references cited. Reversal of the rejection is respectfully requested.

B. There is no reasonable expectation of success based on Shimomura et al., Sode, and Amersham that would lead one of skill in the art to Appellants' invention.

Even assuming that the references could be combined, which Appellants do not concede, there is nothing to provide one of skill in the art any expectation of success in making the alleged combination.

There is nothing in the prior art that would lead one of skill in the art to predict combining the elements as in Appellants' claim, much less predict such excellent effects as are demonstrated by Appellants' claimed method. As noted above, such unpredictability is acknowledged in the June 13, 2006 Office Action, in that the art of obtaining a desired activity in protein chemistry is rather unpredictable (see e.g. page 7, 12-17).

Further, it is Appellants as noted above, who found that using an ion exchange resin containing a quaternary ammonium group in the column with an eluent containing cholate obtains excellent results in the purification of glucose dehydrogenase derived from *Burkholderia*.

In fact, Appellants have shown that excellent purification can be obtained by their method, for example, through purified proteins with higher final specific activity. See "Discussion on the Results" at page 27, line 12 to page 28, line 8 of the Specification. For Example, Appellants have demonstrated that using an eluent containing a cholate with the particular column profile, i.e. ion exchange resin containing a quaternary ammonium group, provides higher final specific activity of the protein than when other non-cholate containing eluents are used. See e.g. Examples 1-4 compared to Comparative Examples 1-2 using sodium chloride. For instance, Appellants have shown that the specific activity of the protein is often higher than 1000 U/mg (Examples 2 and 3) and at least as high a 1500 U/mg in some cases (Example 4), which is double and triple the specific activity without using the combination of packing agent and eluent as claimed. Indeed, the Comparative Examples employing sodium chloride as an eluent to purify glucose dehydrogenase derived from *Burkholderia* was known in Sode, in which

the reference goes no further to teach using other eluents, much less the goal of obtaining such high specific activities that can result from the claimed invention. Such results as reported by Appellants would have been unexpected to one of skill in the art.

Examples 1-4 further show that when the particular cholate comprises sodium cholate, as recited in claim 7, such purification benefits are realized. (See e.g. Examples 1-4 compared to Comparative Examples 1 and 2, and results reported in Tables 2-7 and Figs. 1-3.) For similar reasons as discussed with respect to claim 1, there is no reasonable expectation of success for one of skill in the art to arrive at claim 7.

For the foregoing reasons, Appellants respectfully contend that a reasonable expectation has not been shown in the references cited that would lead one of skill in the art to Appellants' claimed invention. Claims 1, 7, and the remaining dependents are patentable over the references cited. Reversal of the rejection is respectfully requested.

Appellants submit that the rejection is untenable for the reasons set forth above and should be reversed. Please charge any additional fees or credit any overpayment to Deposit Account No. 50-3478.

Respectfully submitted,

Hamre, Schumann, Mueller & Larson, P.C.
P.O. Box 2902-0902
Minneapolis, MN 55402
Phone: 612-455-3800

Date: March 31, 2010

By _____
Name: Douglas P. Mueller
Reg. No. 30,300

Customer No. 52835

VIII. CLAIMS APPENDIX

1. A method for purifying a target protein from a protein solution containing the target protein by using liquid chromatography, wherein the target protein is glucose dehydrogenase derived from a microorganism belonging to the genus Burkholderia and has α, β, γ subunits, the liquid chromatography comprising:

a first step of introducing the protein solution into a column filled with a packing agent, the packing agent holding the target protein, the packing agent being an ion-exchange resin containing a quaternary ammonium group as an ion-exchange group; and

a second step of eluting the target protein by using an eluent containing a cholate.

6. The method for purifying protein according to Claim 1, wherein the β subunit of the glucose dehydrogenase provides electron transfer activity and has a molecular weight of approximately 43 kDa in SDS-gel electrophoresis under a reducing environment, and

the α subunit of the glucose dehydrogenase provides glucose dehydrogenation activity and has a molecular weight of approximately 60 kDa in SDS-gel electrophoresis under a reducing environment.

7. The method for purifying protein according to Claim 1, wherein the cholate comprises a sodium cholate.

8. The method for purifying protein according to Claim 1, wherein the cholate in the eluent is maintained at a constant concentration during the elution of the target protein from the packing agent.

9. The method for purifying protein according to Claim 8, wherein the concentration of the cholate in the eluent is selected from a range of 0.5 through 2.5 wt%.

11. The method for purifying protein according to Claim 1, wherein the microorganism is Burkholderia cepacia KS1 strain (FERM BP-7306).

12. The method for purifying protein according to Claim 1, wherein the glucose dehydrogenase is produced by a transformant, the transformant being produced by engineering a host microorganism with DNA from a microorganism belonging to the genus Burkholderia encoding the α, β, and γ subunits.

13. The method for purifying protein according to Claim 12, wherein the host microorganism is Pseudomonas putida.

14. The method for purifying protein according to Claim 12, wherein the host microorganism is E. coli bacterium.

24. The method for purifying protein according to Claim 1, wherein the α and γ subunits of the glucose dehydrogenase provide glucose dehydrogenation activity and the γ subunit has a molecular weight of approximately 14 kDa in SDS-gel electrophoresis under a reducing environment.

25. The method for purifying protein according to claim 1, wherein the first step using the ion-exchange resin is performed in a non-acidic condition.

26. The method for purifying protein according to claim 1, wherein the first step using the ion-exchange resin is performed at pH 8.

IX. EVIDENCE APPENDIX

N/A

X. RELATED PROCEEDINGS APPENDIX

N/A